Atoh1 (Math1) is a basic helix–loop–helix (bHLH) transcription factor that specifies components of the proprioceptive pathway system in mammals. Despite genetic evidence (knockout mice and overexpression systems) implicating Atoh1 in defining the dorsal interneuron 1 (dI1) population of the dorsal neural tube, developing granule cells in the cerebellum, hair cells of the inner ear, and Merkel cells, precisely how Atoh1 functions in the generation of these cell types remains ill-defined due to the lack of known targets of Atoh1 transcription activity. To identify downstream targets of Atoh1, we performed microarray analyses of isolated Atoh1 populations. Comparison of the Atoh1 populations to other isolated bHLH populations revealed several genes enriched and potentially downstream of Atoh1 in the various proprioceptive lineages. These genes range in identity from small GTPases to tyrosine kinase receptors. By determining the regulation of such downstream targets by Atoh1, we expect to provide insights into how transcriptional networks instruct the generation of specific neuronal lineages.

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Program/Abstract # 150
Using zinc finger nucleases for targeted modification of the zebrafish genome

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Until recently, reverse genetic strategies in zebrafish for targeted mutagenesis have been unavailable. Publications from several groups, including our collaborative work with Sangamo Biosciences, have shown that zinc finger nucleases (ZFNs) can target specific loci for mutation in zebrafish. ZFNs are a fusion between zinc finger protein motifs, designed to recognize and bind to specific DNA sequences, and the nonspecific cleavage domain of the FokI endonuclease. The resulting ZFN binds and cleaves DNA at the target sequence, creating a double strand break (DSB) that must be repaired. One pathway for DSB repair is non-homologous end joining (NHEJ), an error-prone mechanism that introduces small insertions and deletions during repair. In this way, ZFNs have mutagenized 8 different loci in zebrafish. Another application of ZFNs is targeted gene modification utilizing the other DSB repair pathway, homology directed repair (HDR). HDR uses homologous sequence, such as the sister chromatid or an exogenously supplied donor sequence, as a template to direct repair of the DSB. We are currently using *no tail* (*ntl*) targeting ZFNs together with donors to knock-in a small molecular tag or GFP coding sequence into the ntl locus by HDR. We are also using a donor with wildtype sequence and *golden* targeting ZFNs to rescue the *golden*^{b1} point mutation pigmentation defect. Because NHEJ is favored over HDR as the DSB repair pathway choice in zebrafish embryos, we are also examining how manipulation of proteins involved in these pathways may bias the choice towards HDR. We will present our results at the meeting.

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Program/Abstract # 151
Transcriptional regulation of the *c-myb* gene in zebrafish embryogenesis

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The transcription factor c-Myb plays an essential role in stem/progenitor cell proliferation and differentiation in the blood, the brain and the intestine. It is also known to be expressed in other tissues, incl. the retina and the olfactory placode. Little is known about the transcriptional regulation of the *c-myb* gene. This is why we are investigating the transcriptional regulation of *c-myb* in zebrafish embryos. In zebrafish embryos, *c-myb* expression is detected in the retina, the intestine, the olfactory placode, the branchial arches, the brain and in haematopoietic tissues. To find regulatory elements controlling *c-myb* expression in zebrafish we have used three different approaches. Firstly, we have tested sequences upstream of the zebrafish *c-myb* gene. These were amplified by PCR and cloned in front of a *gfp* reporter gene in a *Tol2* transposon vector. The resulting constructs were injected into zebrafish embryos to generate transgenic lines. Secondly, we have searched for regulatory elements outside the promoter proximal region. Assuming that such elements may be evolutionally conserved we searched for conserved noncoding elements in interspecies genomic comparisons using the sequence alignment programme MLAGAN. These elements were individually cloned in front of a basal promoter of *c-myb* in the *Tol2* reporter vector and tested in transgenic zebrafish. Finally, we have modified a BAC which contains 176 kb of genomic sequence around the *c-myb* locus by introducing an *rfp* reporter gene into the *c-myb* gene. The modified BAC has been injected into zebrafish embryos. At the meeting, we will report on the progress we have made.

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Program/Abstract # 152
Characterization of a conserved element at the telomerase promoter

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Telomeres are regions of repeated nucleotides that cap the ends of linear eukaryotic chromosomes. They function as disposable safeguards to prevent the loss of genetic information from end to end fusions, degradation, and instability. Telomerase (TERT) is the enzyme that adds new telomeric repeats to the ends of chromosomes. During embryogenesis, TERT is active and is critical for telomere elongation. In normal human somatic cells, the TERT gene is suppressed after embryogenesis to control cell differentiation and to limit the proliferative capacity of cells. We are investigating the molecular mechanisms which regulate expression of the TERT gene. We analyzed the non-coding genomic region around the human TERT gene using bioinformatic analysis and identified a TERT ultraconserved (TUC) sequence. This 308 bp region is over 75% conserved between distantly related mammalian species and over 91% conserved among primate species. We tested the cis-regulatory potential of the TUC region in a dual luciferase reporter gene assay. Transient transfection into HeLa and lung fibroblast cells demonstrated transcriptional enhancer activity from the TUC region, while transient transfection into mouse embryonic stem cells revealed little regulatory activity, suggesting that different mechanisms may govern the expression of TERT in these cells.

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Program/Abstract # 153
Mapping Dlk1 regulatory elements

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